Molecular Cell, *Volume 43*

Supplemental Information

The TFIIH Subunit Tfb3

Regulates Cullin Neddylation

Gwenaël Rabut, Gaëlle Le Dez, Rati Verma, Taras Makhnevych, Axel Knebel, Thimo Kurz, Charles Boone, Raymond J. Deshaies, and Matthias Peter

Supplemental Experimental Procedures

Yeast protein extracts and immunoblotting

Total yeast extracts for immunoblotting were prepared from exponentially growing cells cultured in YPD at 30ºC. Approximately 10^8 cells were harvested, resuspended in 300µl 20% trichloroacetic acid (TCA), and lysed with a FastPrep® instrument (QBiogene) in the presence of 1ml of 0.5mm glass beads (20s, speed 4.5). TCA-precipitated proteins were pelleted and resuspended in 100µl TCA sample buffer (15% Glycerol, 450mM Tris pH 8.8, 1% SDS, 2mM EDTA, 100mM DTT, 0.005% Bromophenol Blue), denatured 5min at 95 ºC and loaded on 5% Tris-Tricine gels for SDS-PAGE. To detect charged Ubc12 (Fig. 4C), TCA-precipitated proteins were resuspended in Urea sample buffer (115mM Tris pH 6.8, 5% glycerol, 8M urea, 4% SDS, 0.005% Bromophenol Blue) containing either 50mM TCEP or 100mM DTT, and loaded onto 12% Laemmli SDS-PAGE gels. Analysis of Rpb1 degradation after UV irradiation was performed as described (Verma et al., 2011).

Genetic screen for factors regulating Rtt101 modification

We designed a genetic screen to identify non-essential genes regulating Rtt101 ubiquitylation and/or neddylation. Briefly, we crossed a *rub1*Δ strain with a collection of haploid yeast knock-out strains (Tong and Boone, 2006), and compared the sensitivity of single and double mutants to 20µg/mL CPT. A yeast strain deleted in a gene required for both Rtt101 neddylation and ubiquitylation should be sensitive to CPT, and deletion of Rub1 should not increase this sensitivity. Conversely, a yeast strain deleted in a gene required only for Rtt101 ubiquitylation should not be sensitive to CPT, and deletion of *RUB1* should render this strain CPT sensitive. We found 95 strains that scored in the first screen, and 201 that scored in the second one, although most double mutants were less CPT sensitive than the *rub1*Δ *ubc4*Δ strain. We assayed Rtt101 ubiquitylation by immunobloting in 178 of the top scoring strains but could not identify a new factor required for Rtt101 ubiquitylation.

Expression and purification of Rub1

BL21(DE3) cells freshly transformed with the expression vector DU32468 (pGEX6P1-Rub1) were used to inoculate 6 x 1 liter auto-induction medium, supplemented with carbencillin. The cells were grown at 37ºC for 5-6 hours until OD600 > 1.5, at which time the temperature was dropped to 20ºC for another 16 hours to allow auto-induction, expression and accumulation of the fusion protein. Cells were collected by centrifugation, resuspended in 200ml 50mM Tris pH 7.5, 250mM NaCl, 0.5% Triton X-100, 1mM EDTA, 1mM EGTA, 1mM DTT, 10µg/ml

Leupeptin, 1mM Pefablok, sonicated and clarified by centrifugation at 4ºC for 20 min at 40000g. The supernatant was incubated for 90 min with 2ml glutathione-sepharose (GE-Healthcare Lifescience). The sepharose was collected by centrifugation, washed 5 times in 10 volumes lysis buffer and twice in 50mM HEPES pH 7.5, 150mM NaCl, 1mM DTT, 0.03% Brij35, and incubated overnight with 0.2mg GST-Prescission protease. Rub1 was collected in the supernatant after centrifugation, concentrated on Vivaspin 6 10kDa filters (Sartorius), and chromatographed on a Superdex 75 XK 16/60 column to separate GST and GST-Prescision Protease. About half of Rub1 was likely aggregated and eluted in the void at 44ml, while the other half eluted at 75ml – 87ml with an apex at 82ml, suggesting that it was monomeric. Monomeric Rub1 was pure as judged by SDS-PAGE and Coomassie staining.

Details of yeast strains and plasmids used in each Figure:

Figure 1: (A) The strains shown in this panel are BY4741 (wt), scGR29 (*rtt101*Δ) and scGR229 (*rtt101*Δ *rub1*Δ). Rtt101 function in scGR29 and scGR229 was complemented with plasmids encoding wild type Rtt101 or the K791R mutant (pGR82 and pGR83 respectively). (B) Protein extracts were prepared from scGR97 (wt, wt), scGR91 (wt, K791R), scGR104 (*rub1*Δ, wt), scGR289 (*rub1*Δ *ubc12*Δ, wt), scGR247 (*rub1*Δ *ubc4*Δ, wt), scGR282 (*rub1*Δ *ubc5*Δ, wt) and scGR250 (*ubc4*Δ, wt). (C) Protein extracts were prepared from scGR104 (*rub1*Δ, wt), scGR91 (wt, K791R), scGR97 (wt, wt) or scGR693 (*rub1*Δ, K791R) transformed with plasmids allowing expression of 9Myc-Rub1 (pGR141) or 9Myc-ubiquitin (pGR140). (D) The strains shown in this panel are BY4741 (wt, wt), scGR91 (wt, K791R), scGR32 (*rub1*Δ, wt), and scGR641 (*ubc4*Δ, wt), scGR532 (*rub1*Δ *ubc4*Δ, wt) and scGR32 (*rub1*Δ *ubc4*Δ, K791R).

Figure 2: (A) Protein extracts were prepared from scGR97 (wt, wt), scGR373 (*dcn1*Δ, wt), scGR250 (*ubc4*Δ, wt), scGR371 (*ubc4*Δ *dcn1*Δ, wt), scGR104 (*rub1*Δ, wt) and scGR124 (*rub1*Δ *dcn1*Δ, wt). (C) Protein extracts were prepared from scGR134 (wt, cdc34-2), BY4741 (wt, wt), scGR905 (*hrt1(I57A)*, wt), scGR732 (*hrt1(C81Y)*, wt) and scGR755 (*hrt1(A51Δ)*, wt). (D) Protein extracts were prepared from scGR97 (wt, wt), scGR91 (wt, K791R), scGR907 (*hrt1(I57A)*, wt), scGR733 (*hrt1(C81Y)*, wt) and scGR757 (*hrt1(A51Δ)*, wt). (E) Crude yeast extracts for *in vitro* neddylation reactions were prepared from scGR247 (*rub1*Δ, *ubc4*Δ, *HRT1*), scGR1068 (*rub1*Δ, *hrt1(I57A)*) and scGR738(*rub1*Δ, *ubc4*Δ, *hrt1(C81Y)*).

Figure 3: (A) Protein extracts were prepared from scGR97 (wt, wt), scGR373 (*dcn1*Δ, wt), scGR865 (*tfb3(C16Y)*, wt), scGR250 (*ubc4*Δ, wt), scGR371 (*ubc4*Δ *dcn1*Δ, wt), scGR626 (*ubc4*Δ *tfb3(C16Y)*, wt), scGR104 (*rub1*Δ, wt), scGR124 (*rub1*Δ *dcn1*Δ, wt), scGR624 (*rub1*Δ *tfb3(C16Y)*, wt) and scGR91 (wt, K791R). (B) Crude yeast extracts for *in vitro* neddylation reactions were prepared from scGR247 (*rub1*Δ, *ubc4*Δ, *TFB3*) and scGR1065 (*rub1*Δ, *ubc4*Δ, *tfb3(C16Y)*). (C) The strains shown in this panel are BY4741 (wt, wt), scGR91 (wt, K791R), scGR32 (*rub1*Δ, wt), and scGR604 (*tfb3(C16Y)*, wt) and scGR668 (*rub1*Δ *tfb3(C16Y)*, wt). (D) Protein extracts were prepared from scGR118 (wt, wt), scGR120 (wt, K688R), scGR526 (*rub1*Δ, wt), scGR1007 (*dcn1*Δ, wt), scGR1008 (*tfb3(C16Y)*, wt), scGR1018 (*tfb3(C16Y) dcn1*Δ, wt). (E) Crude yeast extracts for *in vitro* neddylation reactions were prepared from scGR526 (*rub1*Δ), scGR1070 (*rub1*Δ, *dcn1*Δ) and scGR1069(*rub1*Δ, *tfb3(C16Y)*). (F)

Protein extracts to analyze Rpb1 turnover after UV irradiation were prepared from scGR416 (wt), scGR1057 (*cul3*Δ), scGR1058 (*cul3(K688R)*), scGR1049 (*rub1*Δ), scGR1056 (*dcn1*Δ) and scGR1055 (*tfb3(C16Y)*).

Figure 4: (A) Protein extracts were prepared from scGR97 (wt, wt), scGR91 (wt, K791R), scGR865 (*tfb3(C16Y)*, wt), scGR867 (*ccl1-ts4*, wt), scGR869 (*kin28-ts*, wt), scGR871 (*rad3-ts14*, wt), scGR875 (*ssl1(T242I)*, wt), scGR873 (*ssl2-ts*, wt), scGR878 (*tfb1-1*, wt) scGR883 (*tfb5*Δ, wt) and scGR885 (*rad2*Δ, wt). (B) Protein extracts were prepared from scGR97 (wt, wt), scGR91 (wt, K791R), scGR865 (*tfb3(C16Y)*, wt), scGR916 (*tfb3(C16Y) rri1*Δ, wt) and scGR944 (*tfb3(C16Y) lag2*Δ, wt). (C) Protein extracts were prepared from scGR893 (wt), scGR895 (*rub1*Δ), scGR897 (*rri1*Δ, wt) and scGR899 (*tfb3(C16Y)*, wt). (E) Yeast protein extract expressing Protein A-tagged Tfb3 was prepared from scGR674.

List of primary antibodies used in this study:
-PAP (Sigma P1291, 1/1000) was used to detect Protein A-tagged Rtt101, Cul3, Tfb3, Hrt1 Ssl1, Cln2 and Cdc4.
-HA.11 Clone 16B12 (Covance MMS-101R, 1/3000) was used to detect HA-tagged Ubc12.
-Anti-Cdc53 yN-18, (Santa Cruz sc-6716, 1/500)
-Anti-Cdc4 yN-19 (Santa Cruz sc-6714, 1/500)
-Anti-RNA polymerase II 8WG16 (Covance MMS-126R, 1/3000) was used to detect Rpb1.
-Anti-Actin Clone C4 (Millipore MAB1501R 1/2000).
-Anti-Tubulin (Sigma T6199, 1/1000)

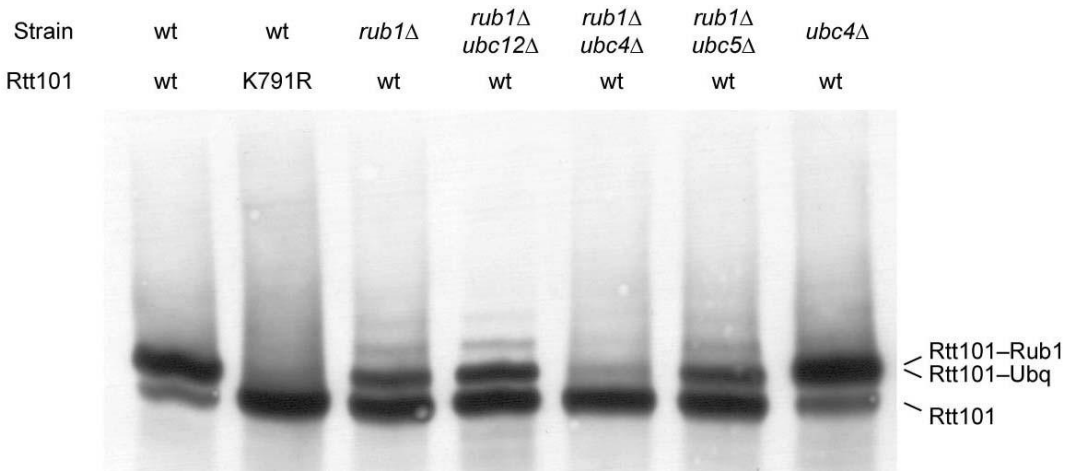

Figure S1. Residual Ubiquitylation of Rtt101 in *rub1*Δ *ubc4*Δ Double Mutant Cells, Related to Figure 1

Immunoblot performed with the same samples as presented in Figure 1B. Note that residual modification of Rtt101 is detectable in *rub1*Δ *ubc4*Δ double mutant cells.

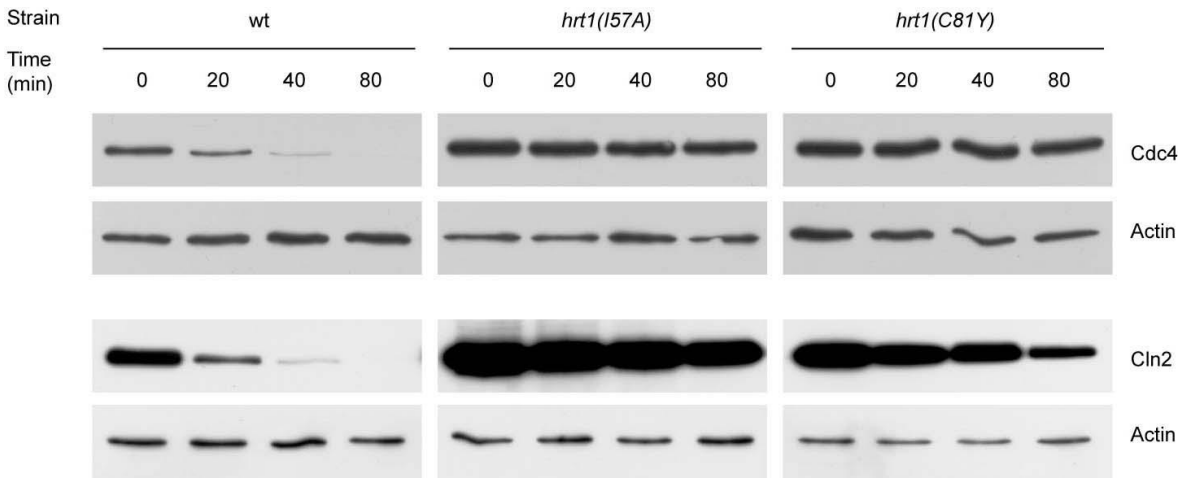

Figure S2. Turnover of Cdc4 and Cln2 in *HRT1* Mutant Strains, Related to Figure 2

To analyze the turnover of Cdc4 and Cln2, cycloheximide was added to exponentially growing cultures of the indicated strains at a final concentration of 100µg/ml. Protein extracts were prepared at the indicated times (min) after cycloheximide addition, and processed for immunoblotting. Actin was used to control equal loading.

Protein extracts to analyze Cdc4 turnover were prepared from BY4741 (wt), scGR732 (*hrt1(I57A)*) and scGR905 (*hrt1(C81Y*) transformed with pGR603. Protein extracts to analyze Cln2 turnover were prepared from scGR1053 (wt), scGR1061 (*hrt1(I57A)*) and scGR1062 (*hrt1(C81Y)*).

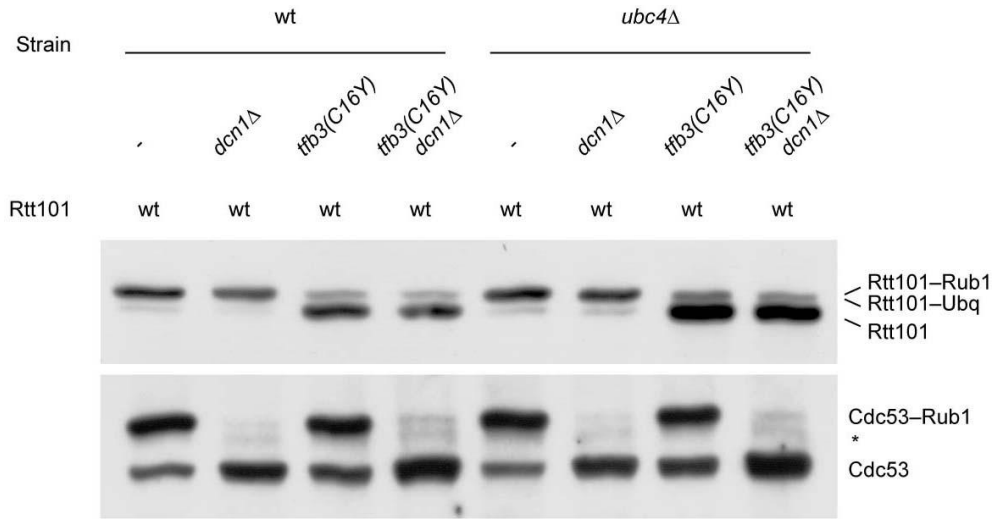

Figure S3. Dcn1 and Tfb3 Do Not Account for Residual Neddylation of Rtt101 and Cdc53 in *tfb3(C16Y)* and *dcn1*Δ cells, Respectively, Related to Figure 3

Immunoblots showing the modification of Rtt101 and Cdc53 in the indicated yeast strains. Protein extracts were prepared from scGR97 (wt, wt), scGR373 (*dcn1*Δ, wt), scGR865 (*tfb3(C16Y)*, wt), scGR977 (*tfb3(C16Y) dcn1*Δ, wt), scGR250 (*ubc4*Δ, wt), scGR371 (*ubc4*Δ *dcn1*Δ, wt), scGR626 (*ubc4*Δ *tfb3(C16Y)*, wt) and scGR979 (*ubc4*Δ *tfb3(C16Y) dcn1*Δ, wt). *: unspecific band.

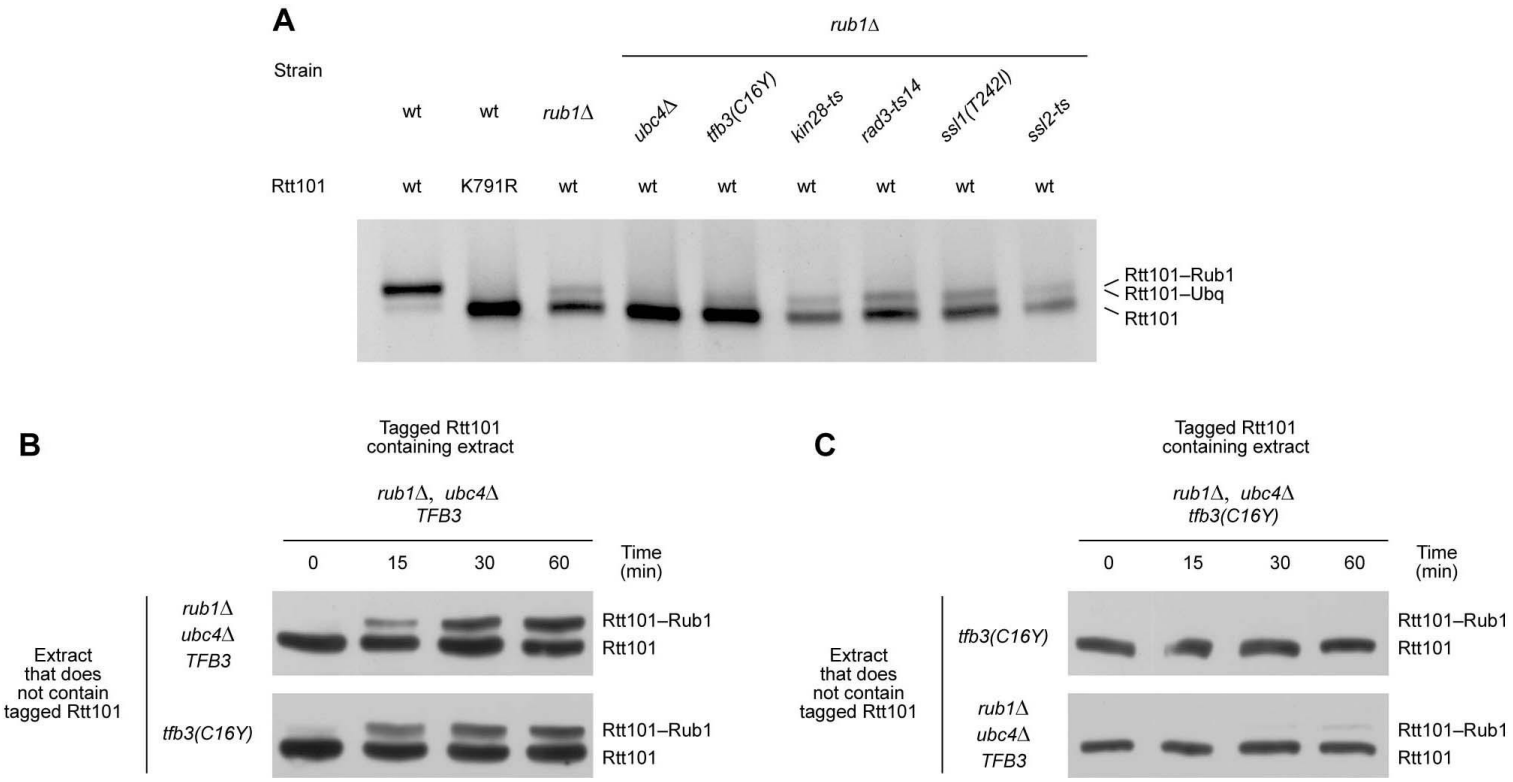

Figure S4. Tfb3-Dependent Regulation of Rtt101 Modification, Related to Figure 4

(A) Mutations in TFIIH subunits other than Tfb3 do not impair Rtt101 ubiquitylation. Immunoblot showing the modification of Rtt101 in the indicated yeast strains. Protein extracts were prepared from scGR97 (wt, wt), scGR91 (wt, K791R), scGR104 (*rub1*Δ, wt), scGR247 (*rub1*Δ *ubc4*Δ, wt), scGR624 (*rub1*Δ *tfb3(C16Y)*, wt), scGR631 (*rub1*Δ *kin28-ts*, wt), scGR632 (*rub1*Δ *rad3-ts14*, wt), scGR635 (*rub1*Δ *ssl1(T242I)*, wt) and scGR637 (*ssl2-ts*, wt).

(B) *tfb3(C16Y)* extracts do not contain a *trans*-inhibitor of Rtt101 neddylation. *In vitro* neddylation was performed in the presence of 5μg/μl of *TFB3* extracts containing tagged-Rtt101 and 20μg/μl of the indicated extracts that do not contain tagged-Rtt101.

The extract containing tagged Rtt101 was prepared from scGR247 (*rub1*Δ, *ubc4*Δ, *TFB3*) and the extracts that do not contain tagged Rtt101 were prepared from scGR287 (*rub1*Δ, *ubc4*Δ) and scGR604 (*tfb3(C16Y)*).

(C) *TFB3* extracts do not rescue Rtt101 neddylation in extracts prepared from *tfb3(C16Y)* cells. *In vitro* neddylation was performed in the presence of 5μg/μl of *tfb3(C16Y)* extracts containing tagged-Rtt101 and 20μg/μl of the indicated extracts that do not contain tagged-Rtt101.

The extract containing tagged Rtt101 was prepared from scGR1065 (*rub1*Δ, *ubc4*Δ, *tfb3(C16Y)*) and the extracts that do not contain tagged Rtt101 were prepared from scGR604 (*tfb3(C16Y)*) and scGR287 (*rub1*Δ, *ubc4*Δ).

Table S1. List of *S. cerevisiae* Strains Used in This Study, Related to Figures 1–4

ID	Genotype	Related to Figure	Ref
BY4741	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0	1A, 1D, 2C, 3C	Brachmann et al., 1998
scGR029	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *rtt101Δ::NAT*	1A	This study
scGR032	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *rub1Δ::NAT*	1D, 3C	This study
scGR091	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-rtt101(K791R)::HIS3*	1B, 1C, 1D, 2D, 3A, 3C, 4A, 4B, S4A	This study
scGR097	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3*	1B, 1C, 2A, 2D, 3A, 4A, 4B, S3, S4A	This study
scGR104	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 rub1Δ::NAT*	1B, 1C, 2A, 3A, S4A	This study
scGR118	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *PATEVCBP9MYC-CUL3::HIS3 ::NAT*	3D	This study
scGR120	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *PATEVCBP9MYC-cul3(K688R)::HIS3 ::NAT*	3D	This study
scGR124	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 dcn1Δ::KAN rub1Δ::NAT*	2A, 3A	This study
scGR154	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *cdc34-2::KAN*	2C	C. Boone lab
scGR229	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *rtt101Δ::NAT rub1Δ::KAN*	1A	This study
scGR247	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 rub1Δ::NAT ubc4Δ::KAN*	1B, 2E,3B, S4A, S4B	This study
scGR250	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 ubc4Δ::KAN*	1B, 2A, 3A, S3	This study
scGR282	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 rub1Δ::NAT ubc5Δ::KAN*	1B	This study
scGR287	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *rub1Δ::NAT ubc4Δ::KAN*	S4B, S4C	This study
scGR289	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 rub1Δ::NAT ubc12Δ::KAN*	1B	This study
scGR371	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 dcn1Δ::NAT ubc4Δ::KAN*	2A, 3A, S3	This study
scGR373	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 dcn1Δ::NAT*	2A, 3A, S3	This study
scGR416	W303 *ADE+ trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-1*	3F	M. Peter lab
scGR526	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *PATEVCBP9MYC-CUL3::HIS3 rub1Δ::NAT*	3D, 3E	This study
scGR532	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *rub1Δ::NAT ubc4Δ::LEU2*	1D	This study
scGR604	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *tfb3(C16Y)::KAN*	3C, S4B, S4C	C. Boone lab
scGR624	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 tfb3(C16Y)::KAN rub1Δ::NAT*	3A, S4A	This study
scGR626	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 tfb3(C16Y)::KAN ubc4Δ::LEU2*	3A, S3	This study
scGR631	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 rub1Δ::NAT kin28-ts::KAN*	S4A	This study
scGR632	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 rub1Δ::NAT rad3-ts14::KAN*	S4A	This study
scGR635	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 rub1Δ::NAT ssl2-ts::KAN*	S4A	This study
scGR637	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 rub1Δ::NAT ssl1(T242I)::KAN*	S4A	This study
scGR641	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *ubc4Δ::LEU2*	1D	This study
scGR668	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *tfb3(C16Y)::KAN rub1Δ::NAT*	3C	This study
scGR674	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *tfb3Δ::KAN* pGR289	4E	This study
scGR687	*MAT*α *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-rtt101(K791R)::HIS3 rub1Δ::NAT ubc4Δ::LEU2*	1D	This study
scGR693	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-rtt101(K791R)::HIS3 rub1Δ::NAT*	1C	This study
scGR732	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *hrt1(C81Y)::LEU2*	2C, S2	This study
scGR733	*MAT*a *ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101::HIS3 hrt1(C81Y)::LEU2*	2D	This study

scGR738	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 rub1Δ*::*NAT ubc4Δ*::*KAN hrt1(C81Y)*::*LEU2*	2E	This study
scGR755	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *hrt1(A51delta)*::*LEU2*	2C	This study
scGR757	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 hrt1(A51delta)*::*LEU2*	2D	This study
scGR865	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 tfb3(C16Y)*::*KAN*	3A, 4A, 4B, S3	This study
scGR867	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 ccl1-ts4*::*KAN*	4A	This study
scGR869	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 kin28-ts*::*KAN*	4A	This study
scGR871	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 rad3-ts14*::*KAN*	4A	This study
scGR873	*MATα ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 ssl2-ts*::*KAN*	4A	This study
scGR875	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 ssl1(T242I)*::*KAN*	4A	This study
scGR878	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 tfb1-1*::*KAN*	4A	This study
scGR883	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 tfb5Δ*::*KAN*	4A	This study
scGR885	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 rad2Δ*::*KAN*	4A	This study
scGR893	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *lys2*-Δ0 *UBC12-HA*::*HIS3*	4C	This study
scGR895	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *lys2*-Δ0 *UBC12-HA*::*HIS3 rub1Δ*::*NAT*	4C	This study
scGR897	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *lys2*-Δ0 *UBC12-HA*::*HIS3 dcn1Δ*::*NAT*	4C	This study
scGR899	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *lys2*-Δ0 *UBC12-HA*::*HIS3 tfb3(C16Y)*::*KAN*	4C	This study
scGR905	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *hrt1(I57A)*::*URA3*	2C, S2	This study
scGR907	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 hrt1(I57A)*::*URA3*	2D	This study
scGR916	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 tfb3(C16Y)*::*KAN rri1Δ*::*NAT*	4B	This study
scGR944	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 tfb3(C16Y)*::*KAN lag2Δ*::*URA3*	4B	This study
scGR977	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 tfb3(C16Y)*::*KAN dcn1Δ*::*URA3*	S3	This study
scGR979	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 tfb3(C16Y)*::*KAN dcn1Δ*::*URA3 ubc4Δ*::*LEU2*	S3	This study
scGR1007	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *PATEVCBP9MYC-CUL3*::*HIS3 dcn1Δ*::*KAN*	3D	This study
scGR1008	*MATα ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *PATEVCBP9MYC-CUL3*::*HIS3 tfb3(C16Y)*::*KAN*	3D	This study
scGR1018	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *PATEVCBP9MYC-CUL3*::*HIS3 dcn1Δ*::*KAN tfb3(C16Y)*::*KAN*	3D	This study
scGR1049	W303 *ADE+ trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-1 rub1*::*TRP1*	3F	M. Peter lab
scGR1053	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *CLN2-TAP*::*HIS3*	S2	Open Biosystems
scGR1055	W303 *ADE+ trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-1 tfb3(C16Y)*::*KAN*	3F	This study
scGR1056	W303 *ADE+ trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-1 dcn1*::*TRP1*	3F	This study
scGR1057	W303 *ADE+ trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-1 cul3*::*KAN*	3F	This study
scGR1058	W303 *ADE+ trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-1 PATEVCBP9MYC-cul3(K688R)*::*HIS3*	3F	This study
scGR1061	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *CLN2-TAP*::*HIS3 hrt1(I57A)*::*URA3*	S2	This study
scGR1062	*MATα ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *CLN2-TAP*::*HIS3 hrt1(C81Y)*::*URA3*	S2	This study
scGR1065	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 rub1Δ*::*NAT ubc4Δ*::*LEU tfb3(C16Y)*::*KAN*	3B, S4C	This study
scGR1068	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-RTT101*::*HIS3 rub1Δ*::*NAT hrt1(I57A)*::*URA3*	2E	This study
scGR1069	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *met15*-Δ0 *PATEVCBP9MYC-CUL3*::*HIS3 rub1Δ*::*NAT tfb3(C16Y)*::*KAN*	3E	This study
scGR1070	*MATa ura3*-Δ0 *leu2*-Δ0 *his3*-Δ1 *PATEVCBP9MYC-CUL3*::*HIS3 rub1Δ*::*NAT dcn1Δ*::*KAN*	3E	This study

Table S2. List of Plasmids Used in This Study, Related to Figures 1–4

ID	Description	Related to Figure	Ref
pGR82	p413adh_PATEVCBP9MYC-RTT101	1A	This study
pGR83	p413adh_PATEVCBP9MYC-rtt101(K791R)	1A	This study
pGR140	p415tef1_9MYC-UBQ	1C	Kraft et al., 2008
pGR141	p415tef1_9MYC-RUB1	1C	This study
pGR289	p415tef1_PATEVCBP9MYC-TFB3	4E	This study
pGR300	pGEX4T1_UBC4	4D	Zaidi et al., 2008
pGR313	pGEX4TG	4D, 4E	Zaidi et al., 2008
pGR339	pGEX4TG_GST-HRT1	4E	This study
pGR345	pGEX4TG_GST-HRT1_Flag-rtt1101(CTD)	4E	This study
pGR495	pGEX4TG_UBC12	4D	This Study
pGR470	pGEX_GST-RAD6	4D	Zaidi et al., 2008
pGR556	pRSFG_PATEV-tfb3(N125)	4D	This study
pGR597	pRSFG_PATEV-ssl1(RING)	4D	This study
pGR598	pRSFG_PATEV-HRT1	4D	This study
pGR603	BG1805_CDC4	S2	Open Biosystems
DU32468	pGEX6P1_RUB1	2E, 3B, 3E, S4B, S4C	This study

More details on these plasmids are available on request.

Supplemental References

Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P., and Boeke, J.D. (1998). Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast *14*, 115-132.

Kraft, C., Deplazes, A., Sohrmann, M., and Peter, M. (2008). Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. Nature Cell Biology *10*, 602-610.

Tong, A.H., and Boone, C. (2006). Synthetic genetic array analysis in Saccharomyces cerevisiae. Methods in Molecular Biology *313*, 171-192.

Verma, R., Oania, R., Fang, R., Smith, G.T., and Deshaies, R.J. (2011). Cdc48/p97 mediates UV-dependent turnover of RNA Pol II. Molecular Cell *41*, 82-92.

Zaidi, I.W., Rabut, G., Poveda, A., Scheel, H., Malmstrom, J., Ulrich, H., Hofmann, K., Pasero, P., Peter, M., and Luke, B. (2008). Rtt101 and Mms1 in budding yeast form a CUL4(DDB1)-like ubiquitin ligase that promotes replication through damaged DNA. EMBO Reports *9*, 1034-1040.